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Adult Plasticity and Rapid Larval Evolution in a Recently Isolated Barnacle Population

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Abstract. *Balanus amphitrite,* a common barnacle species, was introduced into the landlocked Salton Sea in 1943 or 1944. In 1949, *Balanus amphitrite* from the Salton Sea was classified as the subspecies, *Balanus amphitrite saltonensis,* based upon morphological differences between Salton Sea and coastal individuals. This classification was maintained following an investigation of the *Balanus amphitrite* complex in 1975. Such a designation implies that the morphological divergence is underlain by genetic differences. Using field and laboratory transplantations, I tested the alternative hypothesis that the observed morphological divergence in the adult stage of *Balanus amphitrite* was the result of phenotypic plasticity. The results show that the divergence in the examined adult characters is in fact due to environmentally induced phenotypic plasticity. There were also phenotypic differences between larvae from the Salton Sea and those from coastal habitats that only became apparent during experimentation with the adult stage. Here, however, experimental results suggest that the divergence was due to an evolutionary process, probably selection. These results also provide the basis for two slightly precautionary conclusions: (1) the observation that individuals living in typical and novel habitats differ cannot even weakly indicate a cause for the difference, and (2) a consideration of the divergence of populations is incomplete if all of the life history stages of the organism are not studied.

Introduction

One of the continuing challenges in evolutionary ecology is to determine the genetic contribution to phenotypic variation among populations. Two general, and non-independent processes can cause such phenotypic differentiation (Gould and Johnson, 1972; Berven *et al.,* 1979; Falconer, 1989); implicitly assumed in both cases is that, within a species, all individuals share a common ancestor and, therefore, are derived from the same ancestral genotype. First, populations may evolve differently (*evolution*). Second, even when the gene frequencies of two populations do not differ, phenotypic differences may result from plasticity in some traits (*phenotypic plasticity;* see Smith-Gill, 1983; West-Eberhard, 1989 for a discussion of the forms of phenotypic plasticity). The two processes may also interact to produce phenotypic variation among populations.

The distinction between phenotypic differentiation by evolutionary mechanisms and differentiation resulting from phenotypic plasticity cannot be made on the grounds that the latter has a non-genetic basis (West-Eberhard, 1989). Phenotypically plastic responses (to the environment) have as much genetic basis as do other less plastic characters, and plasticity is therefore a trait subject to evolutionary change (Bradshaw, 1965; Williams, 1966; Schlichting, 1986; Macdonald *et al.,* 1988; West-Eberhard, 1989). The distinction is simply that evolution is a characteristic of populations, whereas plasticity is a characteristic of individuals (after Lewontin, 1957). Thus, for populations in which individuals exhibit no plasticity, phenotypic modification as a response to the environment is possible only at the level of the population (across generations). In contrast, for populations in which individuals exhibit plastic characters, phenotypic modification in response to the environment is possible at the level of the individual (within a generation).

Either as untidy noise in complicated genetic systems as it was once regarded (see West-Eberhard, 1989), or as a selectable trait (Schlichting, 1986), plasticity is important to measure. This is because without determining the contribution of phenotypic plasticity, the adaptive significance

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of phenotypic variation cannot be assessed (Berven *et al.*, 1979). In this study I investigated whether evolutionary change or phenotypic plasticity was responsible for the observed phenotypic variation between two Californian populations of the barnacle, *Balanus amphitrite*. One population was from a typical coastal (harbor) habitat in San Diego, California, the other was recently isolated in a novel environment, the Salton Sea.

The Salton Sea is a recently formed landlocked body of saline water, the largest body of water in California. Its average size is 55 by 24 kilometers, but the dimensions vary considerably (Carpelan, 1961a). The genesis and recent history of the Salton Sea have created an environment that is in many ways different from an open marine environment (Carpelan, 1961b) and yet supports a simple, but fascinating, community of introduced marine species. In 1904–1905, a series of floods on the Colorado and Gila rivers breached the headworks of an irrigation channel. For two years these rivers, which normally drain into the Gulf of California, emptied into the Salton Sink, a landlocked sub-sea level basin in southeastern California, and thus formed the Salton Sea. Since 1907, when the headworks were repaired, the level of the Salton Sea has been maintained by irrigation water, and its salinity has risen from 3.65‰ (Carpelan, 1961b) to about 43‰ (Anonymous, 1989); the latter value is between 5 and 8‰ greater than is typical for ocean water. The visibility (usually less than 1 m in the Salton Sea), ionic composition, chlorinity, pH, dissolved oxygen, and temperature fluctuations (10–36°C) of Salton Sea water differ from those of ocean water (Carpelan, 1961b; Raimondi, pers. obs.); because it is an inland body of water, tidal fluctuations in the Salton Sea are trivial.

Balanus amphitrite was first noticed in the Salton Sea in about 1943–1944. Apparently *B. amphitrite* individuals were transported from the San Diego area by air during Naval exercises, as adults on mooring buoys or ropes (Cockerall, 1945; Newman and Abbott, 1980), or as larvae in the bilge water of Naval flying boats (Hilton, 1945). By late 1944 they were ubiquitous: ". . . they were already multiplying so fast that a stick or board only had to be in the water a few days before a crust of minute barnacles started to form." (from Hilton, 1945). "Barnacles now seem to outnumber all other forms of life, both vertebrate and invertebrate, found in the Salton Sea." (from Cockerall, 1945).

In 1949, the Salton Sea population was described as the subspecies *Balanus amphitrite saltonesis* (Rogers, 1949). Subspecific designation was supported by a monograph by Henry and Mclaughlin (1975) on the barnacles of the *Balanus amphitrite* complex; the authors distinguished between *Balanus amphitrite amphitrite* and *Balanus amphitrite saltonensis* on the basis of a multivariate analysis of 15 morphological characters of individuals taken from the field. However, Newman and Abbott (1980) suggested that because the Salton Sea form was also found in a population from Wilmington Harbor (Pacific coast), the difference was ecotypic. Flowerdew (1985) has recently recommended, based upon an electrophoretic investigation of 31 alleles at 11 loci, that the subspecies designation for the Salton Sea population be removed. He found that the values from both indices of *genetic identity* (I) and *genetic distance* (D) were in the range of variation expected between conspecific populations (Nei, 1972). He also concluded that there was no "significant genetic differentiation" of *Balanus amphitrite saltonensis* from *Balanus amphitrite amphitrite*. This implies that no evolutionary divergence could have occurred between the populations, which is incorrect. Nonsignificant I and D values should only be viewed as not refuting the null hypothesis that there is no divergence between populations for the tested alleles (Richardson *et al.*, 1986). Indeed, there are cases of apparently separate (good) species showing no electrophoretic divergence (Avise *et al.*, 1975).

My initial interest was to determine whether the *observed* morphological divergence between Salton Sea and coastal adults (Henry and Mclaughlin, 1975) was due to environmental factors. As the samples used in this initial study came from field collections, there was no way to determine the contribution of the environment to the divergence. In the present study I made no attempt to evaluate any adult character other than those that had been described as differing between the two populations. This was because: (1) I was interested in determining the basis for the differentiating characters, and (2) the selection of additional characters would have been largely unmotivated, because, unlike larvae (see following), when adults from different populations were reared under experimental conditions, they could not be distinguished.

While evaluating the mechanisms determining adult morphological divergence, I found a number of differences in the larvae of the two populations. The basis of these differences was also examined.

Materials and Methods

Study organism, sites, and general methods

Balanus amphitrite is a moderately sized bay barnacle [average basal diameter is between 15 and 20 mm (Newman and Abbott, 1980)], with a virtually world-wide distribution (Henry and Mclaughlin, 1975). Like most thoracican barnacles, it is a simultaneous hermaphrodite (Strathmann, 1987), and fertilized eggs are brooded in the mantle cavity of the parent until they become at least stage one nauplius larvae, when they are expelled into the water. In acorn barnacles such as *Balanus amphitrite*, there are typically seven larval stages (Strathmann, 1987): six naupliar stages (feeding) followed by the final cyprid

stage (non-feeding). All stages are potentially planktonic, but stage one nauplii will often stay within the mantle cavity of the parent, making stage two nauplii the first planktonic stage (Raimondi, pers. obs.).

Adults were collected from three locations: (1) Salton Sea, near North Shore, (2) Mission Bay, California, and (3) Beaufort, North Carolina (larvae from these individuals represented a second coastal population). After collection, individuals were maintained in the laboratory at a water temperature of 20–23°C, and were fed a mixed diet of brine shrimp and the diatom, *Skeletonium costatum* (see Rittschof *et al.*, 1984). As individuals died, new ones were brought in from the field so that 300–500 adults per population were maintained at all times. Adults were induced to expel brooded larvae by a combination of overfeeding and direct bright light. Expelled larvae could then be attracted by a light source and collected.

Larvae were grown in culture at 27–28°C on a diet of *Skeletonium costatum* (see Rittschof *et al.*, 1984, for details of culturing techniques). Larvae from each population were grown in separate containers (usually 3000–5000 per population in 10 l of seawater). The larvae from each of these rearing events were called a batch. Usually, batches of larvae from all populations were reared simultaneously. With this protocol, individuals could not be considered replicates for among-population comparisons, because the effect of batches could not be separated from the effect of populations. Hence, for the examined larval characters, the average value for the individuals within each batch was used as the replicate unit.

All of the larvae used in the experiments described below were reared at the Duke University Marine Laboratory in Beaufort, North Carolina. Upon metamorphosis to the cyprid stage, individuals were collected and shipped live in cold packs, via overnight delivery, to the University of California at Santa Barbara.

Adult characters

As stated, Henry and Mclaughlin (1975) compared the Salton Sea population with coastal populations using a multivariate analysis of 15 morphological characters. The statistical difference between populations was largely due to six ratios of four measurements of the tergum (Table I, Fig. 1). To determine the contribution of environmental factors to the morphological divergence, as manifested in these ratios, I did the following experiment. [The best method of determining whether the morphological divergence between populations was due to environmental differences would have been to reciprocally transplant newly settled individuals from one location to the other (Mission Bay to Salton Sea, and vice versa). Legally and ethically this could not be done].

Cyprids from both the Salton Sea and Mission Bay brood stocks (see above) were allowed to settle on 10×10 cm clay tiles in the laboratory and raised to maturity on those tiles in two environments: "lab," and "lagoon" (the rationale for having two experimental habitats is given below). The density of settlers was about 1 cm^{-2}. Lab individuals were grown under laboratory conditions in the running unfiltered seawater system at the University of California at Santa Barbara. Water temperature during the experiment was about 20°C. Lagoon individuals were raised at the same time as the lab individuals shallow salt water lagoon (approximately 10 hectacres in size) on the campus of the University of California at Santa Barbara, however the water temperature in the lagoon during the experiment varied between 25 and 28°C. The lagoon is separated from the ocean by a sandy barrier through which water, but not plankton, can pass. Salinity in both environments was 32–33% during the experiment. No spontaneous (additional) settlement of *Balanus amphitrite* occurred in either the lab or lagoon.

Lab individuals were fed a mixture of brine shrimp and *Skeletonium* (see above); lagoon individuals fed upon the natural plankton in the lagoon. When the lab and lagoon individuals had grown to 6 to 8 mm basal diameter, they were collected. Individuals of the same size were also collected from both the Salton Sea and Mission Bay; these were the "field populations" in all comparisons. In summary, there were six populations of barnacles: Mission Bay—field, lab, and lagoon; and Salton Sea—field, lab, and lagoon.

From the several hundred individuals reared or collected from each population, 19–54 were randomly and sequentially selected, and from each the tergum was removed and placed individually in a small container of bleach. This procedure removed all tissue from the calcareous mass. Differences in sample size reflect differences among populations in the variability associated with measurements (see Sokal and Rohlf, 1981). Each tergum was drawn using dissecting microscopic and camera lucida projection. From the drawings, measurements of the four tergal dimensions were made and tergal ratios were calculated (Table Ia). Ratios were compared among populations using a multivariate analysis of variance. Ratios were used so the analysis would be comparable to that done in the original work by Henry and Mclaughlin (1975), which described the morphological divergence. However, there are convincing arguments that the use of ratios in morphometric analyses might lead to spurious interpretation of data (Atchley *et al.*, 1976). For this reason, I also compared populations using the four characters (not the ratios, Table Ib) in a multivariate analysis of covariance, as advocated by Atchley *et al.* (1976).

Two experimental habitats were tested because plasticity can be a heritable trait, and the degree of expressible plasticity, if any, might therefore have differed between populations. At the extreme, one population might be

plastic for the examined characters and the other might not be. With only one experimental habitat, there would have been no *a priori* way to control for this possibility. In the following discussion I assume that phenotypic plasticity, if any, will be in the form of phenotypic modulation (Smith-Gill, 1983); this is a reasonable assumption for characters like the ones examined (Table I). Suppose that, in addition to the field populations, there were only the lab populations, and that *a posteriori* analyses indicated: (1) no difference in the examined characters between the Salton Sea and Mission Bay lab populations, (2) no difference between the lab populations and the Mission Bay field population, and (3) that the lab and Mission Bay field populations were all different from the Salton Sea field population. Under this scenario there would be no basis to support the hypothesis that the Salton Sea population was plastic for the examined characters but the Mission Bay population was not, over the alternative hypothesis that both populations were plastic and that lab conditions are similar to conditions in Mission Bay. With the inclusion of a second experimental habitat in the design, the former hypothesis could be ruled out if the Mission Bay lagoon population differed (in the examined characters) from the Mission Bay lab and field populations. If such differences were not observed, then plasticity in the examined characters would not be supported for in the Mission Bay population. A problem could arise if lab, lagoon, and Mission Bay habitats were all similar in the characteristic that induced the plastic response. The test of this would be the comparison of Salton Sea lab and lagoon populations. As the Salton Sea population, in this hypothetical case, was already shown to be plastic, if the examined characters did not differ between the two experimental populations it would suggest that the two habitats were similar in a critical way. Other possibilities concerning the degree of plasticity between populations could be addressed following similar logical steps.

Larval characters

Three characteristics of the cultured larvae differed between the Salton Sea and Mission Bay populations: (1) cyprid pigmentation—Salton Sea cyprids were unpigmented and white, whereas Mission Bay cyprids were greenish-brown, (2) cyprid length—Salton Sea cyprids were larger than those from Mission Bay, and (3) duration in naupliar stages—individuals from the Salton Sea took longer to become cyprids than did individuals from Mission Bay. I did the experiment described below to assess the contribution of environmental factors to the divergence in these larval characters and to correct for a limitation of my initial observations: I did not know if Mission Bay larvae were representative of coastal larvae in general. Although Henry and Mclaughlin (1975) surveyed adult characteristics for a number of *Balanus amphitrite* populations and found that coastal individuals were similar, there have been no comparisons of larval characteristics across populations for this species. Hence, I knew that Mission Bay adults were representative of typical coastal populations for the examined characters, but I had no idea about the scale of phenotypic differentiation among populations of larvae.

I compared cyprid length and pigmentation (color), and duration in the naupliar larval stages, of individuals from the Salton Sea, Mission Bay, and Beaufort, North Carolina, that were reared in a laboratory under identical environmental conditions. Beaufort larvae were compared to ones from Mission Bay to determine the extent of divergence between geographically well-separated coastal populations (*i.e.,* Atlantic *vs.* Pacific populations). Two categories of larvae were used in experimentation: G1 and G2. G1 larvae were progeny of adults brought from the field to the lab and used as brood stock. To minimize the effect of the parental environment, the first release of larvae, which may have developed within the brooding adults in the field, was not used. Some of the G1 larvae were raised to maturity under laboratory conditions and their progeny, G2 larvae, were also examined as a further control of residual parental effects. No G2 Beaufort larvae were cultured because comparisons of Mission Bay G1 and Beaufort G1 larvae indicated that these two coastal populations did not differ for the examined larval characters.

There are two general methods for defining and measuring color (from Chamberlin and Chamberlin, 1980): (1) visual comparison with a standard that is accepted as a reference, and (2) instrumental measurement of the fundamental make-up of the constituent parts of the color in terms of the relative contribution of absorption and reflectance of each wavelength. Both methods were used.

For each batch of cyprids, 2000–3000 from each population were put in separate test tubes (cyprids from each population in one test tube) and chilled to 6°C. This procedure did not damage the larvae, and it caused them to congregate in the bottom of the tubes. The color of the mass of cyprids was then compared to standards contained in the *Methuen Handbook of Colour* (Kornerup and Wanscher, 1978). No statistics are possible for this type of color definition, therefore the Methuen coding will be reported for reference.

To quantify an aspect of coloration, microspectrophotometry was performed on two batches of each larval population. In initial sampling I found that there was divergence in light transmittance between Salton Sea and coastal populations in the range of 450 to 700 nanometers. For logistical reasons I decided to concentrate comparisons on a particular wavelength and chose 510 nanometers. Transmittance was measured through a 40 × 40

Table Ia

Morphological characters used in the multivariate analysis of variance (MANOVA; explanation for the two tests is found in the text). See Figure 1

1) The width of the tergal spur (sw)/the length of the basal margin (bm).
2) The distance from the basiscutal angle to the margin of the spur (aw)/the length of the tergal spur (sl).
3) sl/sw.
4) aw/sw.
5) aw/bm.
6) sl/bm.

Table Ib

Morphological characters used in the multivariate analysis of covariance (MANCOVA). Basal margin (bm) was used as the covariate. See Figure 1

1) The width of the tergal spur (sw).
2) The distance from the basiscutal angle to the margin of the spur (aw).
3) The length of the tergal spur (sl).

μm section in the middle of each cyprid. Light intensity was standardized prior to each measurement.

Cyprid length was measured with a compound microscope and micrometer. The final larval character that was examined, the rate of larval development, required individuals to be drawn from culture and viewed microscopically. This process can damage larvae and potentially can introduce bacteria or ciliates to the culture. To minimize the risk of larval damage or culture contamination, cultures were checked only once each day to determine the developmental stage of the larvae.

Results

Adult characters

Tergal plate ratios or dimensions (Table Ia–b, Fig. 1) for the six populations were compared in MANOVA and MANCOVA procedures and there was a significant difference between populations (Table II). There were no qualitative differences between the results of the two analyses (MANOVA, MANCOVA), indicating that the use of ratios would not, for this data set, lead to spurious interpretations. Comparisons among populations clearly showed where the differences were (Table II). The field populations (Salton Sea *vs.* Mission Bay) were different from each other, as also shown by Henry and Mclaughlin (1975), and were different from all other populations. However, when grown under similar conditions, there was no difference between Salton Sea and Mission Bay individuals: Mission Bay and Salton Sea lab populations were not significantly different, nor were Mission Bay and Salton Sea lagoon populations. Also, the two lab populations (pooled for comparison) were different from the two lagoon populations (also pooled). Examples of the plates can be seen in Figure 2. Particular attention should be directed to the tergal spur (see Fig. 1 for a detailed diagram of the tergum). These results indicate that the phenotypic differences between field populations in the Salton Sea and Mission Bay are the result of phenotypic plasticity and not genetic divergence.

Plasticity itself is a trait that can be selected (Schmalhausen, 1949; Bradshaw, 1965; Schlichting, 1986), and it could be argued that individuals from one of the two locations (Salton Sea and Mission Bay): (1) might not be plastic, or (2) might not be as plastic as individuals from the other location (Schlichting, 1986). If individuals from one location were not plastic for the examined characters, then there would be no statistical difference between lab, lagoon, and field populations. For individuals from both locations, there were highly significant differences among all experimental populations (Table II). Thus, there is no doubt that individuals from both locations are phenotypically plastic. No conclusive answer may be given to the question of whether one population is more plastic than the other because the degree of plasticity in individuals from the two locations was not directly examined. However, the data suggest that individuals from the two locations are similar in their plasticity (in the examined characters) because there were no differences between

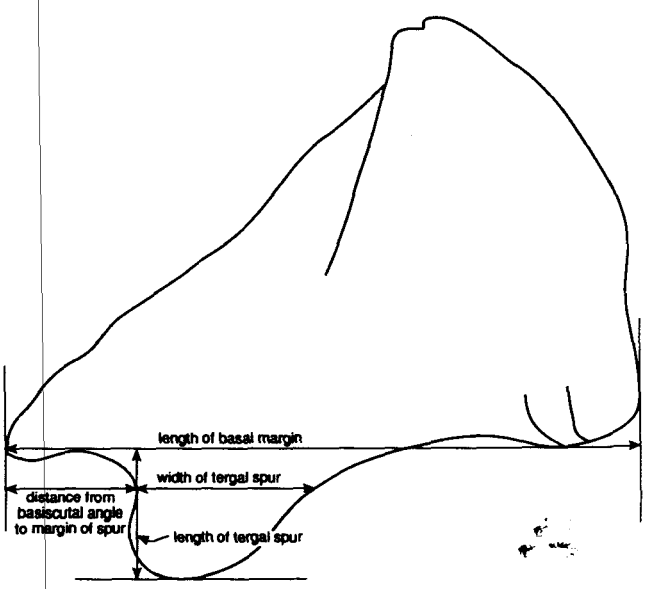

Figure 1. The morphological measurements made on tergal plates: (1) The length of the basal margin, (2) the length of the tergal spur, (3) the width of the tergal spur, (4) the distance from the basiscutal angle to the margin of the spur (see Table I for examined ratios).

Table II

Multivariate (MANOVA & MANCOVA) comparisons of tergal plate measurements between six populations of Balanus amphitrite *(Table 1a–b, Fig. 1)*

	MANOVA		
Pillai trace statistic	DF	F-STAT	*P*-VALUE
1.035	30, 790	6.878	<0.0001
	MANCOVA		
Pillai trace statistic	DF	F-STAT	*P*-VALUE
0.818	15, 474	11.846	<0.0001

	COMPARISONS		
Population comparisons	MANOVA *P*-VALUE	MANCOVA *P*-VALUE	Conclusion
1) Mission Bay field *vs.* Salton Sea field	<0.0001	<0.0001	Populations differ
2) Mission Bay lab *vs.* Salton Sea lab	0.594	0.869	No difference between populations
3) Mission Bay lagoon *vs.* Salton Sea lagoon	0.340	0.247	No difference between populations
4) Mission Bay field *vs.* both lab populations	<0.0001	<0.0001	Populations differ
5) Salton Sea field *vs.* both lab populations	<0.0001	<0.0001	Populations differ
6) Mission Bay field *vs.* both lagoon populations	<0.0001	<0.0001	Populations differ
7) Salton Sea field *vs.* both lagoon populations	<0.0001	<0.0001	Populations differ
8) Both lab populations *vs.* both lagoon populations	<0.0001	<0.0001	Populations differ

Populations: Mission Bay field (n = 26), Salton Sea field (n = 45), Mission Bay lab (n = 28), Salton Sea lab (n = 26), Mission Bay lagoon (n = 21), Salton Sea lagoon (n = 19), both lab populations (pooled n = 54), both lagoon populations (pooled n = 40). Because eight comparisons were made (for each model) the critical P-VALUE for the population comparisons should be 0.05/8 = 0.0063.

reared Salton Sea and Mission Bay individuals in *both* experimental habitats.

Larval characters

For each of the measured parameters, larvae from the Salton Sea differed from the other two populations, which were similar. Differences in pigmentation can result from differences in food type, however, in these experiments food type was constant among populations of parent stock and larvae. The most noticeable difference between Salton Sea cyprids and coastal ones was the lack of pigmentation in the former. Coastal cyprids were consistently green-brown, whereas those from the Salton Sea were white [Number of batches: Salton Sea G1 (8), Salton Sea G2 (2), Mission Bay G1 (7), Mission Bay G2 (2), Beaufort G1 (5)]. As compared to the Methuen color standards (Kornerup and Wanscher, 1978), the color of individuals from the Salton Sea was white (standard 3A1), while that for individuals from either of the coastal populations was olive (standards 3F8–3F4). Individuals from the two coastal populations were indistinguishable on the basis of color. The results from a microspectrophotometric analysis at 510 nanometers substantiated the finding that pigmentation differed between individuals from the Salton Sea and coastal populations (Table III, Fig. 3).

Cyprid length also differed between Salton Sea and coastal populations, which were similar (Table IV, Fig. 4). The third measured parameter was the time between release of larvae by an adult and the metamorphosis from the 6th naupliar larval stage to the cyprid stage (Fig. 5). No analysis was performed on these data as there was no way to meet an often unrecognized shared assumption of parametric and nonparametric statistics: similarity of distributions among groups (Day and Quinn, 1989). Naupliar duration was invariant among all populations except Salton Sea G1, and therefore there is no way to homogenize variance terms. However, it should be obvious without a probability value that naupliar duration was longer for the Salton Sea populations than for the coastal populations.

In all cases where it was examined, within a population there was no statistical difference between G1 and G2 cyprids indicating that residual environmental effects did not affect the results (Figs. 3–5).

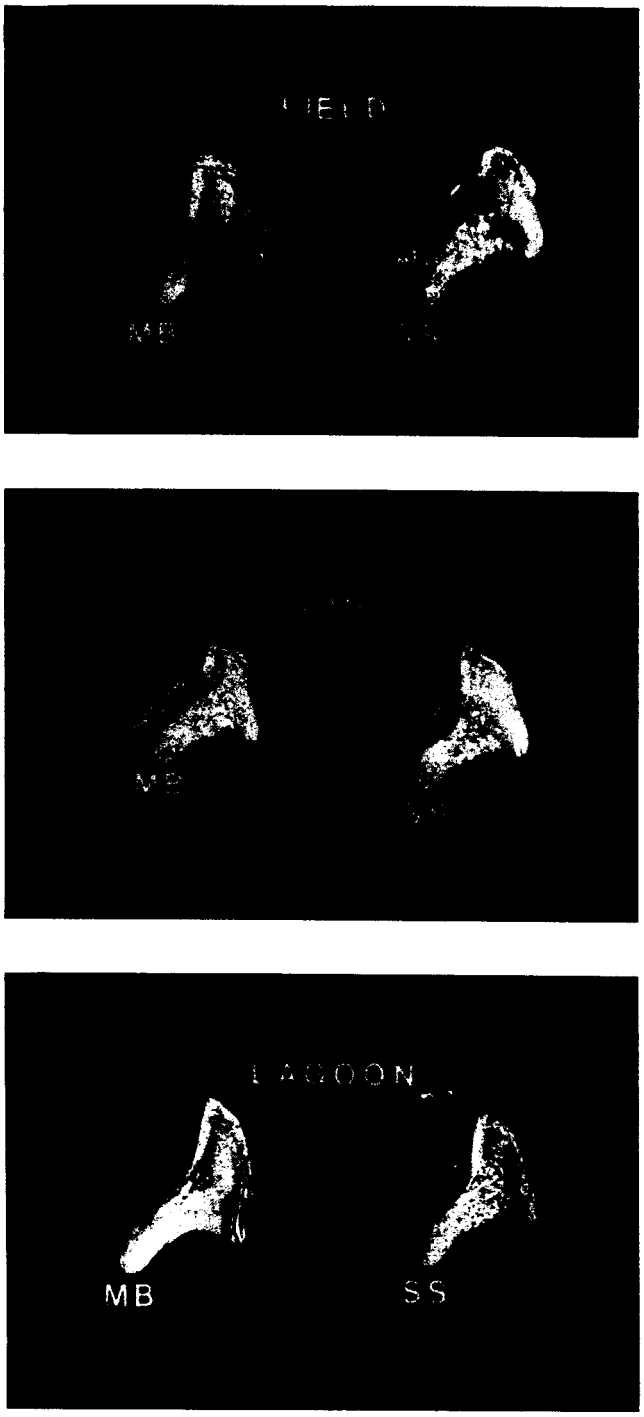

Figure 2. Tergal plates: (Top) Field populations: Mission Bay (left), Salton Sea (right). (Middle) Lab populations: Mission Bay (left), Salton Sea (right). (Bottom) Lagoon populations: Mission Bay (left), Salton Sea (right).

Discussion

The linkage of evolutionary arguments to ecological observations has been rather severely criticized in recent years. These criticisms are in two forms. First, the assignment of specific evolutionary mechanisms to phenotypic divergence has been questioned on the logical grounds that most investigators postulating such mechanisms did not properly test alternative hypotheses (Connell, 1980; Underwood, 1990; but see Roughgarden, 1983). The second criticism has been directed at investigators who failed to consider genetic constraints when proposing evolutionary explanations for ecological data (Gould and Lewontin, 1979; Lande, 1979, 1982; Templeton, 1981; Lynch, 1984). For an examination of phenotypic divergence of an isolated population in a novel environment, like *Balanus amphitrite* in the Salton Sea, understanding these criticisms is crucial because phenotypic modification of individuals in the novel environment is the expected result of *either* evolutionary or plastic processes (see Endler, 1986). Hence, the observation that individuals differ between coastal habitats and the Salton Sea cannot even

Table III

A comparison of transmittance of light at 510 nanometers through cyprids from five populations: (1) Salton Sea G1, (2) Salton Sea G2, (3) Mission Bay G1, (4) Mission Bay G2, and (5) Beaufort G1

ANOVA Source	df	MS	F	*P*
Population	4	38.65	15.28	0.0052
Residual	5	2.53		

For all populations, two batches of cyprids were examined. *A-posteriori* comparisons are shown in Figure 3.

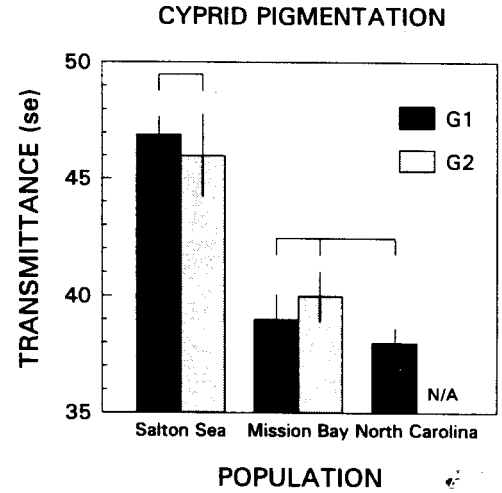

Figure 3. Transmittance of visible light, 510 nm, through cyprids of three populations (no G2 Beaufort cyprids were cultured). Groups not connected by horizontal lines differ at $P < 0.05$ [ANOVA with Tukey procedure, see Table III]. Error bars are ± one standard error of the mean.

Table IV

A comparison of cyprid lengths from five populations: (1) Salton Sea G1 (8 batches), (2) Salton Sea G2 (2 batches), (3) Mission Bay G1 (7 batches), (4) Mission Bay G2 (2 bathces), and (5) Beaufort G1 (5 batches)

ANOVA Source	df	MS	F	*P*
Population	4	1954.16	20.25	<0.001
Residual	19	96.49		

A-posteriori comparisons are shown in Figure 4.

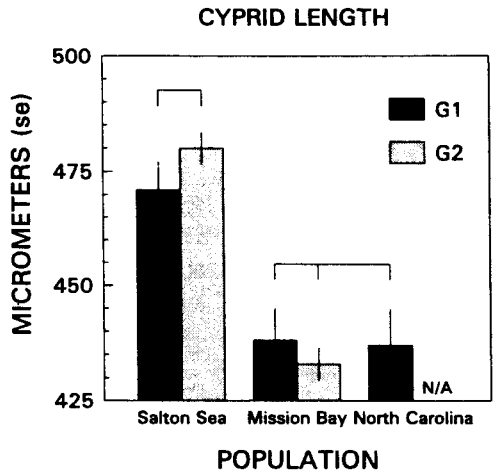

Figure 4. Lengths of cyprids of three populations (no G2 Beaufort cyprids were cultured). Groups not connected by horizontal lines differ at $P < 0.05$ [ANOVA with Tukey procedure, see Table IV]. Error bars are ± one standard error of the mean.

weakly indicate a cause for the difference. The underlying causes for phenotypic divergence in such populations can be determined only through properly designed experiments.

The first goal of this investigation was to determine experimentally if the observed morphological divergence between adult *Balanus amphitrite* in the Salton Sea and those in coastal populations was due to evolutionary or plastic processes. Like Henry and Mclaughlin (1975), I too found that field populations of adult *Balanus* differed for a number of characteristics. However, these differences disappeared when individuals from the two locations were reared in similar environmental conditions (laboratory or lagoon). This is unequivocal evidence that the divergence in the examined characters was due to phenotypic plasticity.

During the investigation of adult characteristics, I found that larvae from the Salton Sea differed from those from Mission Bay. In subsequent experiments, I also found that Salton Sea larvae differed from ones from another coastal population, Beaufort, North Carolina, and that individuals from the two coastal populations did not differ in any examined larval character. The latter result is important because it indicates that widely separated but coastal populations have not diverged for the examined characters. However, it should be noted that coastal populations of bay or harbor species like *Balanus amphitrite* are probably never completely isolated because of transport of adults and larvae by ships (Carlton, 1985). The phenotypic differences between the Salton Sea and coastal populations persisted, undiluted, after two generations in the laboratory, suggesting that the differences are underlain by genetic variation. Genetic crosses are needed to confirm this suggestion (Falconer, 1989), however, *in vivo* crosses would have been confounded by the possibility of self-fertilization (Patel and Crisp, 1961), and *in vitro* crosses that were attempted were unsuccessful.

Assuming that there is a genetic basis for the phenotypic differences found between Salton Sea and coastal larvae, what mechanism may be responsible for the divergence? Only two mechanisms seem plausible: selection and genetic drift, and of these I contend that selection is more likely because there is evidence that there has been no genetic drift. If genetic drift were responsible for the divergence in larval characters for individuals in the Salton Sea then: (1) the Salton Sea population must be isolated from coastal populations, (2) the genes coding for the characters that have diverged must be subject to very little selection (stablizing selection), and (3) the effective population size of the Salton Sea population must have at some time been small (after Falconer, 1989).

If these conditions were all met, then evolution by genetic drift would probably occur. This would likely be

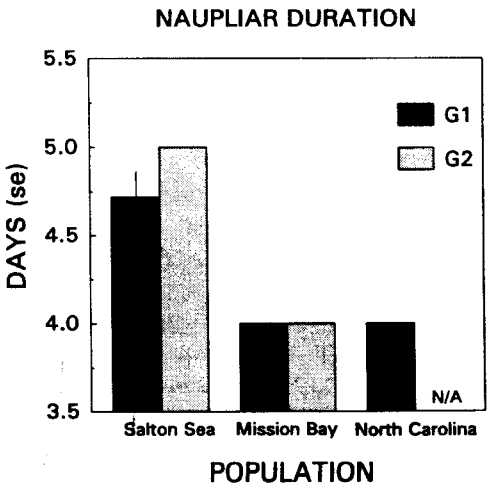

Figure 5. Time between release of larvae from adults and the metamorphosis from the 6th naupliar stage to the cyprid stage (no G2 Beaufort cyprids were cultured). Number of batches: Salton Sea G1 (8), Salton Sea G2 (2), Mission Bay G1 (7), Mission Bay G2 (2), and Beaufort G1 (5). Error bars are ± one standard error of the mean.

reflected in an electrophoretic comparison of allozymes between the Salton Sea and conspecific populations because many of the genes coding for these enzymes would probably be (effectively) selectively neutral (Falconer, 1989). In such a comparison, Flowerdew (1985) found no evidence for either divergence in allelic proportions or loss of heterozygosity for the Salton Sea population of *Balanus amphitrite*. This indicates that significant genetic drift has not occurred in the Salton Sea population. The most likely reason that drift has not occurred is that the inoculation population of the barnacle was not small enough to promote a significant loss of heterozygosity [heterozygosity is lost at a rate of $1/2N_e$ per generation, where N_e = effective population size (Lande, 1980)], and that after the introduction its size increased explosively (Cockerall, 1945; Hilton, 1945).

What selective agents could have caused the divergence of larval characters in Salton Sea *Balanus amphitrite?* As mentioned in the introduction, water in the Salton Sea differs from that in typical oceanic habitats in a number of ways. One that may be important in the present discussion is clarity. Ultraviolet (UV) radiation is harmful to many marine organisms (Jokiel, 1980), and there is a positive relationship between penetration by ultraviolet radiation and water clarity (Jerlov, 1950). Pigmentation has been proposed as an adaptive defense in marine organisms against damage by solar ultraviolet radiation (Ireland and Scheuer, 1979; Yentsch and Yentsch, 1982; Dunlop *et al.,* 1986). I suggest that pigmentation may have been lost by cyprids in the Salton Sea because, in part, the potential for damage by UV radiation in its chronically turbid water is much lower than in coastal water.

The other two characters showing divergence were cyprid length and naupliar duration (the series of naupliar stages constituting the planktonic period prior to metamorphosis to the cyprid stage). Naupliar duration was longer and the resulting cyprids were larger for individuals from the Salton Sea than for those from coastal populations. The increase in cyprid size probably is, at least in part, due to the increase in naupliar period, the period during which larvae feed and grow. In other organisms, larval period is positively correlated with size at metamorphosis, and it has been hypothesized that larval period and the stability of larval habitat should be positively related (Petranka and Sih, 1987; Travis *et al.,* 1987; Newman, 1988). Applied to *Balanus amphitrite,* this hypothesis would require that the Salton Sea be a more stable environment for larvae than coastal bays and harbors. Effectively this would mean that the negative slope of the relationship between larval duration and successful settlement would be less extreme for larvae in the Salton Sea. This seems possible given that predation on larvae, interspecific competition among larvae, maximum distance from hard substrate, and the intensity of storms and currents advecting larvae away from favorable areas for settlement, should all be less for larvae in the Salton Sea.

One of many alternatives to the preceding hypothesis is that longer naupliar periods may be an adaptation to retard a temperature-driven accelerated development rate that would be detrimental to larvae in the Salton Sea. Culture temperature has a dramatic positive effect on the rate of larval development for *Balanus amphitrite* (Rittschof, pers. comm.; Raimondi, pers. obs.), and temperature in the Salton Sea during the period of maximum larval abundance averages between 31 and 36°C (Carpelan, 1961b; Linsley and Carpelan, 1961). I noticed many coastal larvae reared at temperatures above 30°C whose morphology appeared to be intermediate between naupliar stages (Raimondi, unpubl. data). Such larvae have no further development, and their incomplete metamorphoses may result from temperature-driven differences in the maximum rate of development of independent physiological processes. Hence, Salton Sea larvae could show slower development than coastal larvae when reared at 27–28°C because of adaptations to control development at 31–36°C.

Conclusion

The primary aim of this study was to determine whether evolutionary change or phenotypic plasticity was responsible for the observed phenotypic divergence of a population of *Balanus amphitrite* recently introduced and isolated in the Salton Sea. Clearly, divergence in the examined adult characters was due to environmentally induced plasticity. In contrast, there is strong support for the hypothesis that the observed divergence in larval characters was due to an evolutionary process, probably selection. These results are not evidence for a general ontogenetic difference in the way organisms respond to a changing or novel environment. I suspect that there are some unexamined divergent adult traits between populations that are underlain by genetic differences, and some divergent larval ones that are not. However, it is clear that the consideration of divergence between populations is incomplete if all life history stages of the organism are not studied. In the present example I would have found no evidence for genetic divergence between the Salton Sea and coastal populations of *Balanus amphitrite* if only the adult morph had been studied. My final comment is a precautionary one. There has been an historic fascination with examining the causes of phenotypic divergence in isolated populations by considering them as experimental populations. Perhaps this is because they resemble experimental treatments on a larger scale (both temporal and spatial) and with more ecological realism than is possible in manipulations. This is flawed thinking, because there is no

provision for eliminating alternative hypotheses. For *Balanus amphitrite* isolated in a novel environment, the Salton Sea, phenotypic divergence was the expected result of either of two processes: evolution of phenotypic plasticity. Only through experimental manipulations could the responsible process be determined, and then only on a trait-by-trait basis.

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